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have lower estrogen levels than sedentary women. These lower estrogen levels appear to be the mechanism behind their reduced breast cancer risk. Previous studies have included athletes with high exercise levels, and estrogen measurements have been based on a few serum samples taken at different times during a menstrual cycle. We are studying 60 sets of genetically identical female twins who are discordant with regard to moderate exercise levels. Estradiol is being measured on a daily basis by use of salivary samples collected during a complete menstrual cycle. The first year has included development of procedures, questionnaires, and enrollment of eligible pairs. Screening interviews have been conducted with 135 pairs. Sixteen out of 20 pairs of eligible twins have agreed to participate and 10 of these pairs have completed the requirements of the study. Although fewer eligible pairs were identified than expected, additional twins will be available from the second phase of the California Twin Program. In Year 2, we will continue to screen and enroll twins for participation, initiate data entry of questionnaires, and complete laboratory hormonal assays.

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#### A. INTRODUCTION

The purposes of the study are the following:

- (1) To determine the effect of moderate exercise on estradiol (and progesterone levels) over an entire menstrual cycle by means of daily salivary samples in healthy premenopausal identical twins who differ in the amount of physical exercise activity per week.
- (2) To determine the effect of moderate exercise on menstrual cycle length, (specifically luteal phase length among identical twins).

Overview: Exercise has been shown to be associated with a reduced risk of breast cancer. There is evidence that women exercising, for an hour or more per day, have lower serum estrogen (estradiol) levels than sedentary women (due to more anovular cycles and lower estrogen levels in ovular cycles). These lower estrogen levels appear most likely to be the mechanism behind their reduced breast cancer risk, however much is still unknown. Previous studies have, for the most part, focused on the effects of high exercise levels among athletes, as opposed to more moderate levels of exercise, on estrogen levels, and they may have been subject to 'selection bias', i.e. women who exercise may do so because of predisposing hormonal factors. In addition, the estradiol measurements have usually been based on only a few serum samples taken at different times during a menstrual cycle. This study is addressing these issues by using 60 sets of monozygous twins who are discordant with regard to moderate exercise habits (i.e. sedentary vs. exercising an average of 20 minutes/day), but are identical for heritable aspects of body build and constitution. Estradiol is being measured on a daily basis by use of salivary samples collected during a complete menstrual cycle. The subjects are being selected from 426 pairs of healthy premenopausal identical twins under the age of 45 who participated in the California Twin Cohort Study. They are being screened to determine eligibility (i.e. neither twin having an endocrine or metabolic disorder and the pair discordant for current amount of physical exercise activity), before being asked to participate. The use of the salivary samples is an innovative method for the measurement of estradiol and offers distinct advantages over the more traditional serum hormone measurements for which daily samples are not practical. Repeated sampling, as compared to single or infrequent sampling of individuals makes it possible to more accurately characterize ovarian function and allows for a more complete assessment of estradiol levels over different phases of the menstrual cycle, without the discomfort of venipuncture or the inconvenience of office visits. Salivary steroids have been shown to be extremely stable when samples are properly treated and this method of collection is ideally suited for use in the proposed study where subjects are located throughout California. The hormone assays are being done by Dr. Peter Ellison (Co-Investigator), an expert in the analysis of and validation of salivary samples. We are also obtaining information on daily physical exercise activity during the month of sample collection and dietary intake using established and well tested questionnaires. Analysis of covariance methods will be used to assess the relationship of estrogen levels during different parts of the menstrual cycle to exercise, controlling for diet, body mass, and other potentially confounding factors. Based on the sample size of 60 pairs of twins, we have the power to detect differences in estradiol levels of 15% between the sedentary and moderately exercising twins. The study has important public health implications in developing strategies for the prevention of breast cancer.

Background: Hormones and breast cancer risk: A great deal of evidence exists suggesting that ovarian hormones, in particular estrogens, play a major role in breast cancer risk [1-3]. The relationship between age and breast cancer incidence supports this theory. The age-incidence relationship of the common non-hormone related cancers such as colon, stomach and bladder show a continuous steady increase with age. In contrast, breast cancer incidence increases steadily and rapidly with age until about age 50 (average age at menopause) at which time the rate of increase slows dramatically [2]. Direct epidemiological study of the effect of age at menopause shows that for each year a woman's ovaries continue to function there is a 10% increase in her subsequent breast cancer risk [1,2,6]; this is true whether the menopause is natural or artificial (bilateral oophorectomy). The decline in the rate of increase in population incidence around age 50, is thus directly correlated with the markedly reduced serum levels of estrogen (and progesterone) after menopause. Further evidence is provided by the epidemiological observation that women who begin menstruating early have a higher risk of breast cancer than women whose menarche is delayed [1].

Initial reports on estrogen levels in premenopausal breast cancer cases and controls have been inconclusive; however, our recent studies, which paid strict attention to factors which may influence hormone levels in cases, found statistically significant elevated serum levels of estradiol in premenopausal breast cancer cases compared to closely matched controls [7]. The increased levels of estrogen presumably increase risk through their known action as a breast cell mitogen [2]. Luteal phase estradiol (E2) is of major concern, but follicular E2 is also of interest as the breast cell proliferation rate in the follicular phase is some 50% that in the luteal phase and so is a significant contributor to total breast cell proliferation [2]. There is evidence, though controversial, that progesterone also acts to increase breast cell proliferation [2].

Exercise and breast cancer risk: We recently completed a large case-control study of breast cancer cases and healthy controls [4]. Compared to inactive women, risk of breast cancer was reduced by 27% in women who exercised on average 2½ hours per week, and was reduced by 58% in women who exercised 4 or more hours per week (average approximately 60 mins/day), after controlling for established breast cancer risk factors. Our study supports two previous investigations of exercise and breast cancer risk [5,8], and has been confirmed by two subsequent studies [10,11] which also showed a positive association between exercise and lower risk of breast cancer. However, the study based on the NHANES I did not support these findings[9].

Exercise and ovarian function: We believe, based on our understanding of the relation of ovarian hormones to breast cancer risk [2,3], that the observed protective effect of exercise against breast cancer is likely to be due to a reduction in exposure to serum estrogens. Reduced serum estrogen levels may be due to an increased frequency of anovulatory cycles and/or to decreased circulating levels of estrogen in ovulatory cycles. More exposure to estrogen occurs during the luteal phase of the cycle; thus length of this portion of the cycle as well as overall cycle length may also affect level of estrogen exposure.

The prevalence of oligomenorrhea and amenorrhea is higher among athletes than among the general population [12-14]. Studies conducted among athletes and sedentary controls show a consistent association between physically activity and increased frequency of anovulation [15-19]. Only two studies exist which provide data on the effect of moderate exercise activity on the frequency of anovulation. One of these studies is based on only 6 women joggers [16], while the other only included adolescents [20]. These studies also found a higher incidence of anovulation among the more physically active women.

The literature regarding the association of extent of exercise activities and basal levels of ovarian hormones in ovulatory cycles is scant. The only studies with interpretable data are the two studies [18, 21] which confirmed ovulatory status on the basis of an objective serum progesterone measurement. These studies showed that women who exercise have lower levels of circulating estradiol than sedentary controls.

Cycles with long follicular phases are associated with lower than normal cumulative E2 exposure since such cycles have an increased number of days with early follicular phase low E2 levels. Cycles with short luteal phases have also been found to be associated with low E2 values [22].

It has not been established whether cycle length of ovulatory cycles, and in particular luteal phase length, is affected by exercise activity. Three studies [23-25] reported shorter luteal phase lengths in athletes, with no consistent effect on follicular length, but their luteal phase results were not confirmed by other studies [18].

Salivary steroids and ovarian function: Clinical and research applications of salivary steroid assays have spread steadily since their introduction in the late 1970's [26-30]. Salivary steroid levels are considered to reflect biologically active free steroid levels, and so may be functionally more specific than either plasma or urinary steroid determinations. Salivary progesterone measurements have proven particularly useful in the characterization of ovarian function, particularly due to the ease of collecting repeated samples from individual subjects in the field [28, 31-35]. Salivary progesterone profiles have been found to correspond closely to urine and plasma profiles [36], and to coordinate temporally with the timing of the LH surge [37] and the rupture of the dominant follicle [38, 39]. Standards of salivary progesterone levels have been established for both clinical [37, 40-42] and epidemiological [39, 43] applications.

Highly sensitive assays have also been developed for the detection of estradiol in the saliva [27, 43-45]. Salivary estradiol profiles demonstrate a close temporal relationship to salivary progesterone profiles and are highly correlated with plasma levels [45, 46].

### Summary of work done during the first year:

1) Developed screening questionnaire and general questionnaire

2) Assembled the Study Kit which includes the introductory letter, informed consents, the saliva collection tubes and associated materials (including instructions, gum, pen, log sheet), questionnaires (general, diet, and daily activity logs), and return mailing supplies into a box for mailing to each participant.

3) Identified identical premenopausal female twins discordant for exercise from the

California Twin Program.

4) Conducted telephone interviews with selected twins ask screening questions to determine eligibility.

5) Mailed kits to eligible pairs and received completed questionnaires at USC and saliva samples at Harvard University.

The above tasks are consistent with Technical Objectives 1-4 as listed below:

Technical objectives 1-4: Selection of twins and collection of saliva samples: Ongoing throughout Yrs. 1, 2, and during the first 6 months of Year 3.

- 1. During the course of the study identical female twins will be selected who previously participated in the California Twin Cohort and indicated that they are premenopausal.
- 2. These pairs will called on the telephone and re-interviewed regarding factors related to their eligibility.
- 3. Once a pair is determined to be eligible and they agree to participate they will be mailed informed consent forms, saliva sample collection kits, and exercise and dietary questionnaires.
- 4. We will check with them periodically to determine when the first day of their period occurs and assure that they are following the directions for collection of the saliva samples.
- 5. They will mail their completed sample kits to Dr. Ellison's laboratory and the completed questionnaires to USC.

With regard to Technical Objective 5, the completed kits have been sent to Harvard and the first batch of hormonal assays will be completed in the near future.

Technical Objective 5: Completion of Hormonal Assays: Year 1, month 3 through Year 3, Month 9.

- 1. Dr.Ellison's Laboratory will receive the kits and will be blinded as to which twin is performing more exercise.
- 2. The laboratory assistant will complete the hormonal assays according to standard protocols.
- 3. Results will be sent to USC.

No work has yet been done on Technical Objectives 6-7, but data entry and dietary analyses of the first batch of questionnaires will be done shortly.

Technical Objectives 6-7: Data Management: Year 1, Month 6-Year 3 Month 10

- 1. Physical Activity questionnaires will be coded and entered at USC.
- 2. Dietary questionnaires will be sent to Dr. Willett for analysis, with results being sent to USC.

3. Hormonal assay data will be merged with the questionnaire data.

Work has not yet begun on Technical Objectives 8-9.

Technical Objectives 8-9: Data analysis and publishing of papers: Year 1, Month 12-Year 3, Month 12.

- 1. Preliminary and final analyses will be performed to address the stated hypotheses.
- 2. Papers will be published on the results.

Specific Description of Work Completed:

### **Questionnaire Development**

Screening Questionnaire: The screening questionnaire (Appendix 1) was designed to determine if both twins currently meet the eligibility requirements for the study which include:

- 1) both twins are still premenopausal and have had regular periods in the last 6 months,
- 2) both twins were parous or both twins were nulliparous.
- 3) neither have had breast, ovarian, uterine, or cervical cancer,
- 4) neither have been diagnosed with an endocrine or metabolic disorder,
- 5) neither have taken oral contraceptives, hormone replacement therapy, or steroid medications of any type in the past 6 months,
- 6) neither have been pregnant or lactated within the past year, and
- 7) they differ in their exercise levels

This screening questionnaire is administered over the telephone. Once both twins have been interviewed a determination is made regarding the pair's eligibility for the study based on both twins' responses. If eligible, both members are called back and asked to participate, and if they agree, they are then sent the Study Kit.

General Questionnaire: The general questionnaire was finalized and is included in the Study Kit (see below) (Appendix 2). It obtains information on physical activity, current weight, height, reproductive history, use of infertility drugs and oral contraceptives, family history of cancer, smoking and alcohol use, and background information. The participants fill out the questionnaire and mail it back to us along with other study materials.

### **Contents of Study Kit:**

The following items are included in the Study Kit and mailed in a box by priority mail (at a cost of \$4) to each participant:

- 1) Introductory Letter (Appendix 3)
- 2) Informed Consent (Appendix 4)
- 3) Saliva Donation form (Appendix 5)
- 4) General Questionnaire (Appendix 2)
- 5) 40 copies of Physical Activity Daily Log (Appendix 6)
- 6) Willett Diet Assessment form (and #2 pencil) (Appendix 7)
- 7) Saliva collection materials (supplied by Dr. Ellison's laboratory at Harvard) including:

- a. 40 saliva collection tubes (with ID numbers) in plastic container
- b. Sharpie pen for writing dates, initials, and time of collection on tubes
- c. Carefree chewing gum to assist with saliva flow
- d. Collection instructions (Appendix 8)
- e. Saliva collection log sheet (Appendix 9)
- 8) Pre-addressed and pre-stamped (\$3 priority mail) brown return envelope for mailing of signed Informed consent, saliva donation form, general questionnaire, diet assessment, and physical activity logs to USC.
- 9) Pre-addressed and pre-stamped (\$3 priority mail) cardboard return box for mailing of the saliva collection tubes and saliva collection log to Harvard.

## Identification of Female Identical Twins for Screening and Screening Results

Initially 180 pairs of identical female twins from the California Twin Program were selected for screening based on the following information derived from the questionnaire they completed in 1992:

- a. they were both premenopausal (and had not had a hysterectomy) and under 45 in 1992.
- b. neither reported having had breast, ovarian, uterine, or cervical cancer, diabetes, asthma, or Graves disease. In addition, neither were on kidney dialysis, or taking insulin, oral diabetes medicine, or prednisone or other steroids.
- c. their total minutes of exercise reported per week differed by at least 20 minutes and one of the twins was sedentary.

We did not exclude twins if they said they had taken oral contraceptives in 1992 at the time of the original questionnaire, because we wanted to ask them about their current use of these drugs during the screening interview since their use may have changed during the interim.

### Results of Screening interviews

To date, both twins from 135 of the 180 originally identified pairs have been interviewed over the telephone to determine their eligibility. The results are shown in Table 1.

At this time, 16 eligible pairs are participating in the study. Reasons given by those 4 pairs of twins who were eligible, but one member refused to participate, mainly included being too busy. Since we are asking that the individual participant collect a saliva sample every day for approximately 30 days and complete questionnaires, it is understandable that some may not wish to participate. However, of those who have said yes, we have had no drop outs.

We are still making an effort to locate the lost pairs and are continuing to screen the remaining 45 pairs originally identified. An additional 246 pairs are available to contact from the California Twin Program; however, they did not differ in exercise level in 1992. Since changes may have occurred in the interim, they may be eligible at this time. A more promising source of eligible pairs is from a new phase of the California Twin Program. In June, new questionnaires were mailed to approximately 35,000 twins who are in the age group 10 years younger than the original group. We are confident that we will lose fewer pairs to menopause in this new group and their exercise and hormone use information will be current; hence eligible pairs will be more likely to be identified directly from the questionnaire and less time will need to be spent screening pairs.

These questionnaires are in the process of being scanned and data from them will be available before the end of the year. Thus we feel confident that we be able to identify enough additional eligible pairs to complete the original sample size requirements of 60 pairs over the 3 year grant period.

Table 1: Results of Screening Interviews

Result of Screening of Both	Number of	Percent of Total
Members of Pair	Pairs Pairs Screened	
Eligible and consented	16	10.5
Eligible and refused	4	3.0
Screened and not eligible because:	93	68.9
1+ had menopause	(12)	( 9.0)
1+ had very irregular periods	(2)	(1.5)
Parity discordant	(19)	(14.3)
1 + had disqualifying disease	(5)	(3.8)
1+ taking OC's or hormones	(31)	(23.3)
1+ taking cortisone/prednisone	(5)	(3.8)
1+ breast fed a child or		
pregnant within past year	(4)	(3.0)
Multiple of above reasons	(8)	(6.0)
Both had same exercise level	(7)	(5.3)
Lost, could not screen	15	11.3
Refused screening interview	7	5.3
Total	135	100.0

### Completion of Study Requirements by Eligible Pairs

Study Kits have been mailed to the 16 eligible pairs (32 individuals) who agreed to participate. To date, we have received completed questionnaires and saliva collection tubes back from both members of 10 of these pairs. The remaining 6 pairs are currently in the process of collecting the samples as they were mailed the Kits within the past 2 months. One pair decided that it would be too much to do after receiving the Kit, but upon further conversation with them, it was determined that they didn't object to collecting the saliva samples, but didn't want to complete all of the accompanying questionnaires. We waived the daily physical activity log, and eliminated some of the questions in the general questionnaire, and then they were willing to participate.

#### **Laboratory Procedures**

The samples from the 10 pairs have been received at Harvard and they are currently evaluating the samples prior to performing the assays. A saliva collection log form was added after about half of the Kits had been mailed, and we are currently calling some twins to resolve some ambiguities about the days of menstrual flow. One twin missed collecting saliva on several days during her

cycle. It might be necessary to ask her to repeat the collection. Otherwise, the samples appear to have been collected according to the instructions given.

#### C. CONCLUSIONS

The first year of the study has included development of study procedures, questionnaires, and coordination of work with Dr. Ellison's Laboratory. Screening interviews have been conducted with 135 pairs. In general, the eligible twins who agreed to participate (16 pairs so far) are willing to complete the rather demanding requirements of the study; however we had fewer pairs that were eligible than anticipated. Also, we found that a saliva collection log form needed to be added to the Study Kit and this procedure has been implemented. Additional twins will soon be available for screening and participation due to the implementation of the second phase of the California Twin Program (funded by other sources). Thus, these initial problems are not seen as problematic. In the next year, we will continue to screen and enroll twins for participation, data entry of questionnaires will be initiated, a batch of completed Willett dietary assessments will be sent for analysis, and we expect to have the laboratory hormonal assays completed on all pairs who have participated. Procedures will be developed for merging the data from these various sources into a unified record.

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### E. APPENDICES

- 1. Screening Questionnaire
- 2. General Questionnaire
- 3. Letter to participants
- 4. Informed consent
- 5. Saliva Donation Form
- 6. Physical activity daily log sheet
- 7. Diet Questionnaire
- 8. Saliva collection instructions
- 9. Saliva collection log form

## APPENDIX 1 Screening Questionnaire

Answers for Twin 1 (Seqno=1)

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IDI AIN	

## CALIFORNIA TWIN PROGRAM EXERCISE STUDY SCREENING QUESTIONNAIRE Answers for Twin 2 (Seqno=2)

Date: 1) First of all, to verify our information, what is your birthdate? Month/Day/Year Month/Day/Year 2) Have you ever been pregnant? 1. No (Skip to Q.3) 1. No (Skip to Q.3) 2. Yes 2. Yes If yes: A. How many live born children have you had? Number Number B. Have you been pregnant at any time during the past year? 1. No (Skip to Q. 3) 1. No (Skip to Q.3) 2. Yes: What was the 2. Yes: What was the outcome? outcome? 1. Livebirth Livebirth 2. Miscarriage/Abortion 2. Miscarriage/Abortion 3. Stillbirth 3. Stillbirth 4. Other:\_\_\_\_ 4. Other:

	What was the date that your baby was born (or pregnancy ended)?	What was the date that your baby was born (or pregnancy ended?)
	m/d/y	m/d/y
3) Have yo	u breast fed a baby in the past year?	The second secon
	1. No 2. Yes: When did you stop?	1. No 2. Yes: When did you stop?
	m/d/y	m/d/y
prepara A.	the past year, have you taken any of ations? Oral contraceptives, (including birth norplant implants)?	the following hormone containing control pills, depo-provera shots, or
	1. No 2. Yes: What did you take?	1. No
	(If unsure ask: Do you hav Can you get	the bottle or pill container? the information and I will call you back?)
	In what month did you last take them?	In what month did you last take them?
B. Horm	Month: one Replacement Therapy pills such	Month:as Premarin, or the patch?
	1. No 2. Yes: What did you take?	1. No 2. Yes: What did you take?
	(If unsure ask: Do you had Can you ge In what month did you last take them?	we the bottle or pill container?  It the information and I will call you back?)  In what month did  you last take them?
1	Month:	Month:

acne, asthma, or inflammation?	
1. No	1. No
2. Yes: What did you take?	2. Yes: What did you take?
(If unsure ask: Do you have the	ne hottle or pill container?
(If unsure ask. Do you nave as Can you get the	information and I will call you back?)
In what month did	In what month did
you last take them?	you last take them?
Month:	Month:
Control of the Contro	months?
Have you had a menstrual period in the last 6	
1. No	1. No 2. Yes (Skip to Q. 6)
1. No 2. Yes (Skip to Q. 6)	2. Yes (Stap to Q. b)
was the month and year when	If no: What was the month and year when
f no: What was the month and year when you had your last period?	you had your last period?
	/
mo yr.	mo. yr.
The state of the s	Why did your periods stop?
Why did your periods stop?	
1. Natural menopause	1. Natural menopause
2. Surgery (hysterectomy)	2. Surgery (hysterectomy)
	3. Other:
(Skip to Q. 14)	(Skip to Q.14)
•	in the second of
6) When did your last menstrual period start?	
month/day/year	month/day/year
	The modiat
7) Overall, would you say that your periods are	e regular? (that is, you can usually predict
about when the next period will start)?	
about when the next period will start):	1 No (Skin to (), 9)
about when the next period will start)? 1. No (Skip to Q. 9)2. Yes	1. No (Skip to Q. 9)2. Yes

8) About hov	w many days do you usually have betwee L the first day of bleeding) and the first d	lay of the next period? Would you
	e the first day of biodesia,	
say:	1. Less than 20	1. Less than 20
		2. 20-24
	<u></u>	3. 25-29
	3. 25-29	4. 30-34
	4. 30-34	5. 35+
	5. 35+	6. Can't predict
	6. Can't predict	Can't predict
9) How man	y periods have you had in the last 6 mon	ths?
	Number	Number
10) Have yo	ou experienced hot flashes in the past year  1. No 2. Yes	r? 1. No 2. Yes
	2. Tes	<del></del>
	ze a When did you first	If yes: When did you first
	If yes: When did you first	experience them?
	experience them?	
		1
		mo. yr.
	mo. yr.	
	About how often do you have them?	About how often do you have them?
	Numberper	Numberper
	(day, week,	(day, week
	month)	month)
swimm person	e past year, did you participate in any reging, aerobics, bicycling, tennis, weight-lift breathe hard?  1. No (Skip to Q. 12)  2. Yes	gular exercise such as jogging, cting, or other activities that make a  1. No (Skip to Q. 12)  2. Yes
If yes: A.	In what activities do you (did you) partic	ipate?
-		
В	. When did you start?	
		mo. yr.
	· mo. yr.	mo. J.

C. Are you still exercising now?	
1. No: When did you stop?	1. No: When did you stop?
month	month
	2. Yes
2. Yes	
D. How many times per week do you (did yo	ou) usually participate in regular
exercise?	
	N. Jan
Number	Number
	wine internals) Would you say:
(If can't give an answer, prompt with the follow	1 Once
and the second of the second o	2 2-4
	3. 5-7 3. 3-7
3. 5-7	4. 8-10
4. 8-10	5. 11 or more
5. 11 or more	
perform the exercise each time?  Number of minutes	Number of minutes
(If can't give an answer, prompt with the follo	muing intervals) Would you say:
(If can't give an answer, prompt with the joint	1. Under 15 minutes
1. Under 15 minutes	2. 15-29 minutes
2. 15-29 minutes 3. 30-59 minutes	3. 30-59 minutes
4. 60 minutes or more	4. 60 minutes or more
) In the past year, did you participate in any ot	her regular exercise such walking, golf
2) In the past year, did you participate in any of gardening, and other forms of exercise that us	
1. No (Skip to Q. 13)	1. 110 (bing to g)
2. Yes (2.1 de la 1997)	2. Yes
If yes: A. In what activities do you (did yo	ou) participate:
this type of everrie	ing now?
B. Are you still doing this type of exercisi	
1. No: When did you stop?	1. No: When did you stop
I. No. When did you stop.	D. ART A
**	
month	month

G. The second times to	oer week do vou (did you)	usually participate in regular	
C. How many times percise?	Jet week do jou (= " )		
		Normalian	•
Number		Number	
	with the followin	a intervals) Would you sav::	*. *******
(If can't give an answe	r, prompt with the jollowin	g intervals) Would you say::  1. Once	. •
1. Once		2. 2-4	
2. 2-4	ene number of	3. 5-7	•
3. 5-7	•'	4, 8-10	
4. 8-10	0.00	5. 11 or more	•
5. 11 or m			
D. When you exercis	se(d), about how many mi	nutes, on average, do (did) you	
perform the exerc	cise each time?		
Number of mir		Number of minutes	강 기업 생각 등 시간 현실 시간 시간 시간 일본 사람들은 기업 시간 시간 시간 시간 기업
	ar prompt with the following	ng intervals) Would you say:	
(If can't give an answ1. Under 1	5 minutes	1. Under 15 minutes	
2. 15-29 r	ninutes		
3. 30-59 r	minutes	3. 30-59 minutes	
	utes or more	4. 60 minutes or more	٠.
ever told you that you h that you had:	and a condition I have me	nditions. Please stop me if a doc ntioned. Has a doctor ever told	``````````````````````````````````````
Ovarian cysts	1. No2. Yes	1. No2. Yes1. No2. Yes	
Polycystic ovaries, or Stein Levanthal Syndrom	1. No2. Yes	1. NO2. 165	
Diabetes, Sugar diabetes	1. No2. Yes	1. No2. Yes	<u>-</u> .
or high blood sugar			
-	1, No2. Yes	1. No2. Yes	e and all of the first section of the section of th
Any of the following thyro	oid diseases:		• • • •
Grave's Disease	1. No2. Yes	1. No2. Yes	
Hashimoto's disease or chronic thyroiditis	1. No2. Yes	1. No2. Yes	
Overactive thyroid (hyperactive)	1. No2. Yes	1. No2. Yes	:

Underactive thyroid (hypoactive)	1. No2. Yes	1. No2. Yes
Any type of Cancer?	1. No2. Yes	1. No2. Yes
What type (site)?		
How old were you: when first diagnosed?		
14) Have you ever had a	mammogram?	
1. No 2. Yes		1. No 2. Yes
Who	en was the last time?	When was the last time?
mo		mo. yr.

Those are all the questions I have for you. Thank you very much for your time and effort. We will be reviewing your answers as well as those of your twin and will be calling you back to let you know if you and your twin are eligible.

## APPENDIX 2 General Questionnaire

USC/Norris Comprehensive Cancer Center University of Southern California Department of Preventive Medicine 1441 Fastlake Ave, Mail Stop 44 Los Angeles, California 90033-0800



Name:	•	
Study Number:	· · · · · · · · · · · · · · · · · · ·	and the second of the second o

An Innovative Assessment of Endogenous Estrogen Activity in Persons with Different Habits of Exercise

**General Questionnaire** 

**Any Questions?** 

Please call and leave a message at our Toll Free Number (1-800-421-9631), or you may call the Principal Investigators directly: Dr. Ann Hamilton at 213-764-0434 (after June 13 the number will be 323-865-0434), or Dr. Lisa Shames at 213-764-0422 (after June 13, 323-865-0422). Our FAX number is 213-764-0141 (323-865-0141 after June 13). Dr. Hamilton can also be reached by e-mail at ahamilt@hsc.usc.edu.

Physical Activity Profile:	Please fil	l in today's d	late:		_/ay Year
Please list any sport or recreation you h frequently you have participated in it, a Please remember to include seasonal s	s indicate	d in the tabl	ted in du e.	uring the past	YEAR and
AME OF SPORT, RECREATION OR OTHER PHYSICAL ACTIVITY	per \	ber of WEEKS /EAR you cipate in the ity	WEEK do when acti	TIME spent per ling the activity ve	Total Number of YEARS you have participated
		er volume for the	<u> </u>	<u>-1 </u>	e a jeda e a dijelaci Postava e a
				1	
<u> </u>				1	·
Please list any sport or recreation you he Please remember to include seasonal seaso	ports or e	Number of TIM have participate activity this WE	ES you	Average TIME S	
				1	
				Property In	. 14
				1	
	· .	<u> </u>	·	,	
Approximately how many flights (not nu climb <u>up</u> each <u>day</u> ?	ımbers) o	f stairs do yo	ou usual	ly <i>Fligh</i>	hts
Approximately how many city blocks or walk each day?	their equ	ivalent do yo	ou regula	arly	

STUDY	NUMBER	ξ
-------	--------	---

On a usual weekday and a weekend day, how much time do you spend on the following activities? Total for each day should add up to 24 hours.

	TYPE OF ACTIVITY	Usual Week Day HOURS PER DAY	Usual Weekend Day HOURS PER DAY
a.	Vigorous activity (e.g., digging in the garden, strenuous sports, jogging, chopping wood, sustained swimming, bris walking, heavy carpentry, bicycling on hills)	sk	
b.	Moderate activity (e.g., housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing light carpentry, dancing, bicycling on level ground)	9.9	
C.	Light activity (e.g., office work, driving a car, strolling, personal care, standing with little motion)		
d.	Sitting activity (eating, reading, desk work, watching TV, listening to radio)		
e.	Sleeping or reclining		
		Total=24 hours	Total=24 hours
7.	jogging, bicycling, etc. long enough to work up a swethumping, or get out of breath?  What is your usual pace of walking?		1) Yes 2) No
	(Please check one)	1) Casual or strolling 2) Average or norm 3) Fairly brisk (3 to 4) Brisk or striding	mal (2 to <3 mph)  <4 mph)
8.	Do you have a job (either full or part-time)?	1) Yes, full time 2) Yes, part time 3) No	进行 (数 1.5
	8.1 If Yes, How much of the time on your job do y items, or do other physical activities (other tha	ou usually walk, climb st n sitting or standing)?	tairs, lift heavy
	(Please check one)	1) None of the tim2) Less than half the3) At least half the4) Most of the time5) All the time	the time e time

	<b>.</b>	Of a least one in	ile at each workout.	· -	1) Yes 2) No
9.1	A. Please li B. The age C. The age D. The tota E. On aver	when you first be when you stopped all number of years	ou participated in, egan participating, ed, s your participated i ours per week you	n the activity during participated in the a	these ages
A. Name of	Activity	B. Age began	C. Age stopped	D. Total Years	D. Hours per week
1.		1 H 15			
2.				·	
3.					
4.					
5					
10. How tal	I are you with much do yo	u currently weigh	(in pounds)?	Fee	t Inches Pounds (Current)
11. a) How				?	
	much did yo	u weigh at the ag	e of 20 (in pounds)		Pounds (Age 20)
b) How	it has been th	u weigh at the ag ne most you have es when you were	weighed?		Pounds (Age 20)  Highest Weight

·		STUDY NUMBER
14.	Compared to this time a year ago, how much has your weight	1) No change 2) Gained 5 lbs. 3) Gained 6-10 lbs.
		4) Gained 11+ lbs. 5) Lost 5 lbs. 6) Lost 6-10 lbs. 7) Lost 11+ lbs.
15.	How does your hip measurement compare to your waist measurement?	1) Hips much bigger2) Hips bigger3) About the same4) Waist bigger5) Waist much bigger
11.	Reproductive History	
13.	What was your age at your first menstrual period?	
		Age
14.	(That is, you could predict within a day or two when you	to become regular? Ir next period would
	occur) (Please check one)	1) Less than 2 months 2) From 2-6 months 3) From 7-12 months 4) More than a year 5) Never regular
15.	How many times have you been pregnant?	Number
16.	How old were you when you had your first pregnancy?	Age
17.	. What was the outcome of your first pregnancy?	1) Live born child2) Miscarriage/Abortion3) Stillbirth4) Other
18	. How many live born children have you had?	Number

19. How many boys? How many girls?

Number

Number of boys

Number of girls

		STUDY NUMBER
20.	How old were you when you had your first live born child?	Age
21.	How old were you when you had your last live born child?	Age
<b>2</b> 2.	Have you given birth to twins?	1) Yes 2) No
23.	Totaling all periods of breast feeding and all children, how would you estimate that you have breast fed?	1) Never breast fed 2) 1-5 months 3) 6-11 months 4) 12-23 months 5) 24-35 monts 6) 36 or more months
24.	Did you ever consult a physician about a fertility problem	?1) Yes 2) No
	If Yes: What was the cause of the problem?	1) No problem found2) A problem with you3) A problem with your husband4) A problem with both of you5) Never resolved
25	. Did you ever get treatment for an infertility problem?	1) No2) Yes, took fertility drugs3) Yes, had in vitro fertilization4) Yes, other treatment
26	. Have you ever taken oral contraceptives (birth control pills?)	1) Yes 2) No
	If Yes: 26.1 At what age did you first take them? Last take them?	Age First Age Last
	26.2 For how many years altogether have you taken them?	Total Years

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• • •			

## III. Family History

- 27. For each of your relatives listed in the chart below, please fill in the information indicating:
  - A. If they are still living, and if so their current age If not living, their age at death
  - B. If they have ever had cancer, and
  - C. If so, how old they were when it was diagnosed, and where in the body the cancer started.

These questions pertain to your <u>biological</u> relatives only, please do not include adopted, or step relatives. If you don't have sisters (other than your twin) or brothers, leave those spaces blank.

Relative	A. Still Living?	B. Ever had cancer?	C. If Yes
1. Mother	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
2. Father	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
3. Sister #1 (other than twin)	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
4. Sister #2 (other than twin)	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
5. Brother #1	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
6. Brother #2	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
(If additional brothers/ sisters list below) 7	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
8	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:
9	Yes: Current age No: Age at death:	Yes No:	Age diagnosed:

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28. Did either of your grandmothers	have cancer?	
a. Maternal Grandmother	1) Yes: Cancer Site:2) No3) Don't Know	<del>_</del>
b. Paternal Grandmother	1) Yes: Cancer Site:2) No 3) Don't Know	
29. Did any of your aunts (mother's	sisters or father's sisters) have cancer?	
a. Mother's Sisters	1) Yes: Cancer Site(s):	
	2) No 3) Don't Know 4) Mother had no sisters	
b. Father's Sisters	1) Yes: Cancer Site(s):	
30. Did any of your uncles (mother	's brothers or father's brothers) have cancer?	
a. Mother's Brothers	1) Yes: Cancer Site(s):2) No 3) Don't Know 4) Mother had no brothers	<u> </u>
b. Father's Brothers	1) Yes: Cancer Site(s):2) No 3) Don't Know 4) Father had no brothers	
IV. Lifestyle Habits		
31. Have you ever smoked a to	otal of 100 cigarettes or more in your lifetime?	1) Yes 2) No
If Yes: 31.1 How old were you w	hen you first started smoking cigarettes?	Age Started
31.2 Have you smoked in	n the last 6 months?	1) Yes 2) No
If No: At what age of	did you stop?	Age Stopped

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	31.3	How m	any cigarettes	do you (or did you	in the past)	usually smoke?	
				# of cigare	ettes _	1) Per day 2) Per week 3) Per month	
<b>32</b> .	How wou	ıld you d	escribe your c	urrent pattern of di	rinking alcoho	ol?	No. 1
		2]3)45678	Usually have Usually have Usually have Usually have Usually have Usually have	sional drink (less tabout 1 drink per vabout 2-6 drinks per about 7 drinks per about 8-14 drinks about 15-21 drinks more than 21 drinks	week. er week. week. per week. s per week. ks per week.		
33.	How doe	1 2 3 4	No change Drink somewl Drink a lot mo Drink somewl Drink a lot les Drink more no	hat more now than ore now than previ hat less now than as now than previo	previously ously previously usly at some time	ous patterns of drinking	
34.	of alcoh	olic beve	rages?			you) pick each of the for	ollowing types

Туре	1) Never	2) Once in awhile	3) Sometimes	4) Most of the time	5) Always
A. Wine	1)	2)	3)	4)	5)
B. Beer	1)	2)	3)	4)	5)
C. Liquor	1)	2)	3)	4)	5)

35. On how many of the last 14 days did you have a beer, a glass of wine, or any other alcoholic drink?

_1) None
2) 1-3
3) 4-6
4) 7-9
5) 10-12
 6) 13-14

	STUDY NUMBER
	5.1. If 1+: On the days that you did drink, how many drinks per day, on average, did you have? 1) One2) 23) 34) 45) 56) 6-97) 10 or more
<u>V. Ba</u>	ground Information
<b>36.</b>	Vhat is the highest level of formal education you have completed?  (Please check one)  ——1) Less than high school ——2) High school graduate ——3) Some college/trade school ——4) College graduate ——5) Post graduate
37.	Are you currently employed?1) Yes
	2) No2) No2. The state of th
	37.2 Please describe two primary duties of your job?
38.	To what race/ethnic group of Americans do you belong?
39.	Please indicate your total (family) yearly household income?

Please continue and complete the purple Diet Assessment by filling in the circles with a #2 pencil. Thank you.

\_1) Below \$20,000 \_2) \$20,000 - \$29,000 \_3) \$30,000 - \$39,000 \_4) \$40,000 - \$49,000 \_5) \$50,000 - \$59,000 \_6) \$60,000 or more

# APPENDIX 3 Letter to Participants

USC/Norris Comprehensive Cancer Center University of Southern California Department of Preventive Medicine 1441 Eastlake Ave, Mail Stop 44 Los Angeles, California 90033-0800



323-865-0434

FAX: 323-865-0141

email: ahamilt@hsc.usc.edu

September 29, 1998

«Title» «FirstName» «LastName» «Address1» «City», «State» «PostalCode»

Dear «FirstName»,

Thank you very much for agreeing to participate in this study regarding the effect of activities and other factors on estrogen levels in twins. In this box, we have enclosed all the information and supplies you will need for this study including:

- 1) Informed consent for you to sign that lists all the parts of the study that you are asked to participate in (including completion of questionnaires and collection of saliva).
- 2) Saliva donation form for you to sign
- 3) General questionnaire
- 4) Diet Assessment (purple form) with pencil
- 5) Daily physical activity logs (40 copies)
- 6) Saliva collection kit
- 7) Saliva daily log form
- 8) Carefree gum, pen, and instructions for collection of saliva
- 9) Mailing materials to mail back the informed consent, saliva donation form, General Ouestionnaire, Diet Assessment, and daily physical activity logs to us.
- 10) Mailing materials to mail the saliva log form and saliva collection tubes to the laboratory at Harvard University.

First of all, please read and sign the informed consent and the saliva donation form and complete the General Questionnaire and Diet Assessment. On the first day of your next menstrual period, please call us and leave a message at our Twin Study toll free 800 number (800- 421-9631). Ashley Gallagher will be the person you may speak with or you may just leave a message with your name and phone number, and Gayle Alis, our Research Assistant, will call you back.

On the next morning after your period starts, begin collecting the saliva samples. We will be measuring estrogen levels from these saliva samples and special care must be taken when collecting the samples to be sure that the hormone measurements are accurate. The procedure is to collect the saliva before you eat anything (or wait at least 15 minutes after eating (or

smoking)). Also, please do not brush your teeth prior to collection of the sample because brushing may cause your gums to bleed and this can throw off the measurement of the hormone levels. We are asking you to collect saliva every morning from the day this period starts until the first day of your next menstrual flow (about 30 days).

The tubes are marked with your study number; however we ask you to write the date and time of collection on each tube when you collect the sample and also to put your initials on it. On the days when you have menstrual flow, please mark those tubes with an 'M'. Please also record this information on the 'Saliva Daily Log Form'. Chewing the gum we have provided will help to stimulate saliva flow. Please fill the tube to the bottom of the tape. Please do not use any other brand of gum.

The tubes should be kept in a safe place away from children and pets; however they do not require refrigeration or any special conditions. When your time of collection is completed (i.e. your next period has started), please make sure the tubes are tightly capped and mail the tubes to the laboratory at Harvard in the packaging provided. The postage and address label are already on the box; thus all you need to do is to put the container with the tubes in the box, stuff some of the bubble wrap (or a little paper) around the container, seal the box with the tape provided, and put it in the mail.

On the days when you are collecting the saliva samples, we are also asking you to fill out a daily physical activity log at the end of each day. When the sample collection is completed, please insert the informed consent, the saliva donation form, the General Questionnaire, the Diet Assessment, and the daily physical activity logs into the mailing envelope provided (with prepaid postage and address already on it) and put it in the mail to us.

If, at any time, you have any questions or problems please call us on our toll free Twin Study number (800-421-9631) and leave a message. You may also call either of us and leave a message, or we will call you right back to minimize any phone charges to you. Gayle Alis, our Research Assistant, will be calling you to see how things are going.

Thank you again for your willingness to participate in this very important study. We will keep you informed about the results.

Sincerely,

Ann S. Hamilton, Ph.D. Co-Principal Investigator 323-865-0434

Lisa Shames, Ph.D. Co-Principal Investigator 323-865-0422

email: ahamilt@hsc.usc.edu

## APPENDIX 4 Informed Consent

Dr. Ann Hamilton
Dr. Lisa Shames
(323) 865-0434
NorrisCancer Center/USC School of Medicine
1441 Eastlake Ave. MS #44
Los Angeles, CA 90033

# An Innovative Assessment of Endogenous Estrogen Activity in Persons with Different Habits of Exercise

Initials

### Participant Informed Consent

PURPOSE: This study is being conducted in an effort to learn more about the effects of physical activity on ovarian hormones in premenopausal women.

PROCEDURE: Your participation in this study involves three aspects. First, you will be asked to complete a survey regarding your exercise habits, dietary intake and personal medical history. Second, you will be asked to collect 5ml's (approximately 5 teaspoons) of saliva (in a plastic tube) over an entire menstrual cycle (or approximately 30 days) by spitting into a tube. The saliva collection should be done one time per day when you first wake up in the morning. Detailed information on how the saliva samples are to be collected are included in the study packet on a form entitled 'Instruction for Participants'. The tube is pre-treated with a small amount of a bactericidal agent called sodium azide. The sodium azide is at the bottom of each tube and is visible as a small dried residue which adheres firmly to the base of each tube. Please keep tubes out of the reach of small children since this agent is toxic when ingested. Lastly, you will be asked to keep a daily log of your physical activities (we will supply you with these forms) over one menstrual cycle.

The entire study will performed over one menstrual cycles (or about 30 days) and will be done in the convenience of your home. We will mail you a study packet complete with instructions, collection tubes, and surveys. The packet should be mailed to the laboratory upon completion of your participation in the study (we will pre-pay postage).

RISKS: There are no conceivable health risks associated with the collection of one's own saliva. However, care should be exercised when storing tubes in a home with small children, since the bactericidal agent adhering to the bottom of each tube (which keeps the specimens fresh), can be toxic if swallowed.

BENEFITS: There are no direct benefits to you. There is, however, the indirect benefit of knowing that you are contributing to a study that may help us better understand how physical activity affects women's health.

ALTERNATIVES: It is understood that you may choose not to participate in this study and that your decision will not in any manner affect your medical care. This study is designed to assess hormone levels over an entire menstrual cycle and there are no other convenient non-invasive

Initials	

alternatives to this method of sample collection. You are free to withdraw your consent and discontinue participation at any time.

CONFIDENTIALITY: The confidentiality of any information obtained through this study will be maintained by the investigators. Any information which personally identifies you will not be released or disclosed without your written consent except as specifically required by law. Representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as part of their responsibility to protect human subjects in research.

OFFER TO ANSWER QUESTIONS: It is understood that if you have any questions, comments or concerns about the study or the informed consent process, you should contact the Principal Investigators; Dr. Ann Hamilton or Dr. Lisa Shames with the Department of Preventive Medicine, 1441 Eastlake Ave., Los Angeles, CA 90033 ((323) 865-0434). If you have any questions regarding your rights as a study participant, you may contact the Institutional Review Board Office at (213) 223-2340. You will be given a copy of this form to keep.

COERCION AND WITHDRAWAL STATEMENT: Your decision whether or not to participate will not interfere with your medical treatment at this university. You are free to withdraw your consent and to discontinue participation at any time. You may decline to answer any survey questions you wish to.

INJURY STATEMENT: If you should require medical treatment as a result of an injury arising from your participation in this study, the financial responsibility for such treatment will be yours.

NEW INFORMATION: Any new information that is developed during the course of this research which may be related to your willingness to continue or discontinue participation in this study will be provided to you.

## CALIFORNIA LAW REQUIRES THAT YOU MUST BE INFORMED ABOUT:

- 1. The nature and purpose of the study.
- 2. The procedures in the study.
- 3. Discomforts and risks to be expected from the study.
- 4. Benefits to be expected from the study.
- 5. Alternative procedures that might be helpful and their risks and benefits.
- 6. Availability of medical treatment should complications occur.
- 7. The opportunity to ask questions about the study or the procedure.
- 8. The opportunity to withdraw at any time.
- 9. A copy of the written consent from the study.
- 10. The opportunity to consent freely to the study without coercion.
- 11. Statement regarding liability for research-related injury (if applicable).

AGREEMENT: Your signature indicates your decenter information provided above.	cision to participate in this study having re
Signature of Volunteer	Date
Signature of Investigator	Date

Form Valid For Enrollment From

AUG 1 5 1998 To AUG 1 4 1999

Institutional Review Board

Date: July 27, 1998

## APPENDIX 5 Saliva Donation Form

## UNIVERSITY OF SOUTHERN CALIFORNIA CONSENT FORM FOR STUDY ENTITLED: AN INNOVATIVE ASSESSMENT OF ENDOGENOUS ESTROGEN ACTIVITY IN PERSONS WITH DIFFERENT HABITS OF EXERCISE

I,	, voluntarily and freely donate my saliva sample to USC all right, title, and interest to this sample.					
:	·					
Participant's signature						
Participant's name (printed)						
Date of signature						

APPENDIX 6 Physical Activity Daily Log						
lease fill out one copy of this form each uring saliva collection period	day					
uring sunva conceación persea	Today's Date:	// Month Day	Y Year			
aily Log: Physical Activity Profile:		, orkin buy				
Please list any sport or recreation you have par time you were physically active (i.e. actual exe gardening, carpentry, calisthenics, etc).	ticipated in during the rcise time while joggi	past <b>24 Hours.</b> Fing, bicycling, swimi	Please include only the ming, brisk walking,			
NAME OF SPORT, RECREATION OR OTHER PHYSICAL ACTIVITY	Number of TIMES you participated in the activity today	Average TIME spen episode doing the a	ctivity participation in activity today			
1.		/				
2		1				
3.		1				
4.		1				
5.		1				
. Approximately how many flights (not numbe	rs) of stairs did you cl	imb <u>up</u> <b>TODAY</b> ?	Filabo			
. Approximately how many city blocks or their	equivalent did you wa	alk TODAY?	Flights Blocks			
. How much time did you spend on the following	g activities TODAY. 1	otal should add up	to 24 hours.			
TYPE OF AC			HOURS TODAY			
<ul> <li>Vigorous activity (e.g., digging in the garden, wood, sustained swimming, brisk walking, he</li> </ul>	avy carpentry, bicyclii	ng on hills)				
<ul> <li>b. Moderate activity (e.g., housework, light sport mowing, painting, repairing,, light carpentry, c. Light activity (e.g., office work, driving a car, s</li> </ul>	ts, regular walking, go dancing, bicycling on l	olf, yard work, lawn level ground)				
motion) d. Sitting activity (eating, reading, desk work, wa	atching TV, listening to	radio)				
e. Sleeping or reclining			Total=24 hours			
<ol> <li>Did you engage in regular activity akin to brisk get your heart thumping, or get out of breath</li> </ol>	walking, jogging, bic TODAY?	ycling, etc. long end				
7. What was your usual pace of walking TODAY	1) C 2) A 3) Fa	e) asual or strolling (< verage or normal (; airly brisk (3 to <4 r risk or striding (4 m	2 to <3 mph) mph)			

. . APPENDIX 7 Willett Diet Assessment 67890003456789 67890123456789 ID: **5**6089<u>000</u>33456089 1. Do you currently take multiple vitamins? (Please report individual vitamins under question z.) 0023466289 06-9 0023456789 a) How many do you take 2 or less Yes — If yes, per week? 0023466789  $\bigcirc$  3-5 10 or more b) What specific brand do you usually use? Specify exact brand and type 2. Not counting multiple vitamins, do you take any of the following preparations: O Don't a) Vitamin A? 10+ yrs. How many  $\rightarrow \bigcirc$  0-1 yr. 2-4 yrs. years? know O No O Yes, seasonal only lf O 8,000 to O 23,000 IU O Don't O 13,000 to 22,000 IU Yes, What dose → Cless than Yes, most months 12,000 IU O Don't b) Vitamin C? How many  $\rightarrow \bigcirc 0$ -1 vr. 2-4 yrs. 5-9 yrs. 10+ yrs. know vears? () No O Yes, seasonal only If O Don't O 400 to 700 mg O 750 to 1250 mg O 1300 mg. What dose → Cless than per day? Yes, Yes, most months know or more per day? Don't c) Vitamin B<sub>6</sub>? ∩ know 10+ yrs. → O-1 yr. 2-4 yrs. 5-9 yrs. How many years? O 10 to 39 mg. 80 mg. O Don't O 40 to 79 mg ON<sub>o</sub> Less than Yes → If yes, What dose per day? 10 ma Don't d) Vitamin E? Oknow → O-1 yr. 2-4 yrs. 10+ yrs How many years? Less than 100 IU O 100 to 250 IU 300 to 500 IU ○ 600 IU O Don't What dose per day? O No Yes → If yes, or more know Don't () 10+ yrs. e) Selenium? 2-4 yrs. O know → O-1 yr. How many years? O 140 to 250 mcg O Don't 260 mcg. O Less than O 80 to 130 mcg  $\bigcirc$  Yes  $\rightarrow$  If yes, What dose per day? () No 80 mcg. or more know Don't f) Iron? O know 0-1 yr. 2-4 yrs. O 5-9 yrs. O 10+ yrs. How many years? O 201 to O Don't O Less than O 51 to 200 mg () 401 mg O No What dose per day? Yes → If yes, 51 mg. or more Don't g) Zinc? 10+ yrs. 2-4 yrs. → O-1 yr. How many years? know O 25 to 74 mg 75 to 100 mg O Don't ) 101 mg. O No Yes → If yes, What dose per day? Character Less than 25 mg. or more know Don't (Include Calcium in Dolomite.) h) Calcium? Oknow 5-9 yrs. 10+ yrs. • 0-1 yr. O 2-4 yrs. How many years? O 400 to Less than 400 mg. O 901 to 1300 mg () 1301 mg. O Don't Yes → If yes, What dose per day? O No O Beta-Ocod liver Olodine Other (please specify):i) Are there other supple-O Folic acid Carotene ments that you take on O Vitamin D O Copper a regular basis? Please Omega-3 Fatty-acids O Brewer's Yeast **B-Complex** mark if ves: Vitamins 3. For each food listed, fill in the circle indicating **AVERAGE USE LAST YEAR** how often on average you have used the amount Never. specified during the past year. 1-3 per per per per than once week day day day day **DAIRY FOODS** (W) (D) Skim or low fat milk (8 oz. glass) (W)  $\overline{\cap}$ Whole milk (8 oz. glass) (D) (W) Cream, e.g. coffee, whipped (Tbs) 8 **(** Sour cream (Tbs) (W) **(** Non-dairy coffee whitener (tsp.) 0 <u>@</u> Sherbet or ice milk (1/2 cup) (W) 0 Ice cream (1/2 cup)  $\overline{\mathsf{O}}$ (W) 0 (D) O Yogurt (1 cup) (W) (D) O Cottage or ricotta cheese (1/2 cup) 0 (W) Cream cheese (1 oz.) Other cheese, e.g. American, cheddar, etc., plain or as part of a dish (1 slice 0 **@** 0 O Ο 0 O O О or 1 oz. serving) Margarine (pat), added to food or bread; 0  $\circ$ 0 0 0 0 0 O O (W) exclude use in cooking Butter (pat), added to food or bread; Please turn (W) (D) exclude use in cooking

to page 2

Page 2 3. (Continued) Please fill in your average use. Never, 1-3 4-5 during the past year, of each specified food. or less than once per per per per per per mo. day week **FRUITS** per month Raisins (1 oz. or small pack) or grapes **@** 0 Ô Prunes (1/2 cup) 0 O 0 Please try to  $\widecheck{\omega}$ Bananas (1) 0 average your Cantaloupe (1/4 melon) <u>@</u>  $\circ$ (e)  $\overline{\cap}$ seasonal use O of foods over Watermelon (1 slice) <u>@</u>  $\overline{\cap}$ <u>(a)</u> the entire year. 000 (W) Fresh apples or pears (1) 0 For example, if 000 Ŏ a food such as Apple juice or cider (small glass) 8 0 cantaloupe is (W)  $\overline{\bigcirc}$ **(**  $\overline{\cap}$ Oranges (1) eaten 4 times a 000 ŏ 0 <u>(0)</u> week during the Orange juice (small glass) approximate 3  $\widetilde{\otimes}$ <u>(a)</u> Grapefruit (1/2) months that it is **@** O O <u>(0)</u> Grapefruit juice (small glass) O O in season, then  $\widetilde{\omega}$ the average use Other fruit juices (small glass) **(** would be once (W) Strawberries, fresh, frozen or canned (1/2 cup) 0 per week. 0 Blueberries, fresh, frozen or canned (1/2 cup) <u>(0)</u> O O Peaches, apricots or plums (1 fresh, or 1/2 cup canned) Never, 1-3 2-4 5-6 2-3 4-5 6+ per per per per than once mo. week week week dav dav dav dav ) 色 **VEGETABLES** per month Tomatoes (1) (W) 0000 **(** 888 Tomato juice (small glass)  $\overline{\cap}$ **(** 0000 Tomato sauce (1/2 cup) e.g. spaghetti sauce 000000 Red chili sauce (1 Tbs) <u></u> Tofu or soybeans (3-4 oz.) **®** <u></u>  $\overline{\mathsf{O}}$  $\overset{\smile}{\otimes}$ <u>(a)</u> String beans (1/2 cup) <u>(</u>8) 000 <u>(0)</u> Broccoli (1/2 cup) Cabbage or cole slaw (1/2 cup) <u></u> Cauliflower (1/2 cup) 0 <u></u>  $\overset{\smile}{\otimes}$ Brussels sprouts (1/2 cup) 0 <u>@</u> Carrots, raw (1/2 carrot or 2-4 sticks) <u></u>  $\widetilde{\otimes}$ Carrots, cooked (1/2 cup) <u></u> O (W) Corn (1 ear or 1/2 cup frozen or canned) <u>(a)</u> <u></u> Peas, or lima beans (1/2 cup fresh, frozen, canned) (e) 000 Mixed vegetables (1/2 cup) 0000 (W) ℗ <u>@</u> Beans or lentils, baked or dried (1/2 cup) **©** <u>O</u> <u></u> Yellow (winter) squash (1/2 cup) **(0**)  $\overline{O}$ **(** Eggplant, zucchini, or other summer squash (1/2 cup) Yams or sweet potatoes (1/2 cup) 000 (W) **(** 00 (W) 0 Spinach, cooked (1/2 cup) **(0**)  $\overline{\bigcirc}$ (W) <u>(a)</u> Spinach, raw as in salad 000 <u>@</u> Kale, mustard or chard greens (1/2 cup) 0 Iceberg or head lettuce (serving) (W) <u>@</u> Romaine or leaf lettuce (serving) <u>@</u> (D)  $\overline{\mathsf{O}}$ Celery (4" stick) <u>@</u> <u></u> (W) Beets (1/2 cup) **(** ŏ  $\overline{\mathsf{O}}$ (S) Alfalfa sprouts (1/2 cup) **(D)** O Garlic, fresh or powdered (1 clove or shake) (W) **(0**) Never, 1-3 2-4 5-6 2-3 4-5 6+ per per per per per per than once day mo. week week week day day day EGGS, MEAT, ETC. Eggs (1) <u>@</u> <u></u> Chicken or turkey, with skin (4-6 oz.) (W) ℗ Chicken or turkey, without skin (4-6 oz.) (W) 0 0 ℗ O O O

O

(W)

0

<u>(0)</u>

Please go to page 3

Bacon (2 slices)

Hot dogs (1)

ark Reflex® by NCS											
3. (Continued) during the	Please fill i past year, o	n your <u>average</u> use, if each specified food.	Never, or less than once	1-3 per	1 per week	2-4 per	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
		MEATS (CONTINUED)	per monti			week					
		Processed meats, e.g. sausage, salami, bologna, etc. (piece or slice)	0	0	<b>∞</b>	0	0	0	O	0	0
		Liver (3-4 oz.)	ㅣᅌ	18	<u>  @</u>	<u>Q</u>	0	<u> </u>	0	<u>Q</u>	<u>Q</u>
		Hamburger (1 patty)		10	<u>@</u>	<u>Q</u>	0	0	0	0	<u>Q</u>
		Beef, pork, or lamb as a sandwich or mixed dish, e.g. stew, casserole, lasagne, etc.	0	0	W	0	0	(e)	0	0	0
		Beef, pork, or lamb as a main dish, e.g. steak, roast, ham, etc. (4-6 oz.)		0	W	0	0	(e)	0	0	0
		Canned tuna fish (3-4 oz.)	0	0	(W)	0	0	0	Q	0	0
		Dark meat fish, e.g. mackerel, salmon, sardines, bluefish, swordfish (3-5 oz.)	0	0	8	0	0	0	0	0	0
		Other fish (3-5 oz.)	0	0	00	0	0_	0	0	0	0
		Shrimp, lobster, scallops as a main dish		0	(W)	0	0	0	0	0	0
			Never, or less than once	1-3 per	1 per	2-4 per	5-6 per	1 per	2-3 per	4-5 per	6+ per
		BREADS, CEREALS, STARCHES	per monti		week	week	week	day	day	day	day
		Cold breakfast cereal (1 cup)	O	Ŏ	8	<u>O</u>	Ö	0	Ŏ	Ŏ	0
		Cooked oatmeal (1 cup)		10	<u>@</u>	<u>Q</u>	<u>Q</u>	<u> </u>	l O	<u> </u>	Ŏ
		Other cooked breakfast cereal (1 cup)		10	<u>@</u>	<u>Q</u>	0	0	0	0	<u>o</u>
		White bread (slice), including pita bread		10	<u>@</u>	l O	0	<u>(</u>	0	0	<u>Q</u>
		Dark bread (slice)	10	10	<u>@</u>	0	<u>Q</u>	0	0	0	$\bigcirc$
		English muffins, bagels, or rolls (1)	18	18	<u>@</u>	0	0	90	0	0	0
		Muffins or biscuits (1)	10	0	8	0	9	0	8	0	0
		Brown rice (1 cup)		18	8	Ö	8	0	8	0	8
		White rice (1 cup) Pasta, e.g. spaghetti, noodles, etc. (1 cup)	18	18	8	0	0	0	0	ŏ	ŏ
			18	18	8	0	ŏ	0	0	ŏ	ŏ
		Other grains, e.g. bulgar, kasha, couscous, etc. (1 cup)					- i				
		Pancakes or waffles (serving)	10	10	<u> </u>	0	<u> </u>	0	l S	8	<u>Q</u>
		French fried potatoes (4 oz.)	10	0	<u>@</u>	0	O.	$\sim$	0		l O
		Potatoes, baked, boiled (1) or mashed (1 cup)	ᆜᅌ	10	<u>  @</u>	0	l S	0	18	<u>  Q</u>	$\frac{9}{2}$
		Potato chips or corn chips (small bag or 1 oz.)	무용	은	<u>@</u>	10	8	<u> </u>	l 은	18	
		Crackers, Triskets, Wheat Thins (1) Pizza (2 slices)	-   응	18	(W)	18	18	0	용	9	00
	i	Pizza (2 slices)	10	10	1 🐷						
	1	BEVERAGES	Never, or less than once per month		1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
ARBONATED		Low calorie cola, e.g. Tab with caffeine	10	10	<b>⊗</b>	0	0	0	0	0	0
BEVERAGES	Low Calorie	Low calorie caffeine-free cola, e.g. Pepsi Free	18	Tŏ	Ö	ŏ	ŏ	õ	ŏ	ŏ	ŏ
onsider the	(sugar-free) types	Other low calorie carbonated beverage, e.g. Fresca, Diet 7-Up, diet ginger ale	ŏ	ŏ	8	ŏ	ŏ	Õ	ŏ	ŏ	ŏ
erving size		Coke, Pepsi, or other cola with sugar	10	0	(W)	0	0	0	0	0	0
as 1 glass, bottle or can for these	Regular types (not sugar-	Caffeine Free Coke, Pepsi, or other cola with sugar	Ö	0	00	Ō	0	0	Ō	Ō	Ō
arbonated everages.	free)	Other carbonated beverage with sugar, e.g. 7-Up, ginger ale	0	0	00	0	0	0	0	0	0
BE	OTHER VERAGES	Hawaiian Punch, lemonade, or other non- carbonated fruit drinks (1 glass, bottle, can)	0	0	00	0	0	0	0	0	0
		Decaffeinated coffee (1 cup)	0	0	(W)	0	0	0	0	0	0
		Coffee (1 cup)	0	0	8	0	0	0	0	0	0
		Tea (1 cup), not herbal teas	0	0	(W)	0	0	0	0	0	0
		Beer (1 glass, bottle, can)	0	0	(W)	0	0	0	Ŏ	Q	Q
		Red wine (4 oz. glass)	10	10	⊚	lõ	Ö	0	Ö	Ö	Ŏ
		White wine 14 oz glass)									

Liquor, e.g. whiskey, gin, etc. (1 drink or shot)

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ID:							(6) (6)	) () ()	900 900	)034 )034	(B) (G)	) (9 (9 (9
3. (Continued) Please fill in your				J 🗸	~		<b>J</b> (	Ď®(	9)			
average use during the past year,	Never,	1-3	1	2-4	5-6	1	2-3	4-5	6+		000	000
of each specified food.	or less than once	per	per	per	per	per	per	per	per		① ® (	
SWEETS, BAKED GOODS, MISCELLANEOUS	per mont	mo.	week	week	week	day	day	day	day		@ @ @	
Chocolate (bars or pieces) e.g. Hershey's, M&M's	0	0	<b>₩</b>	0	0	0	0	0	0		3 6	
Candy bars, e.g. Snickers, Milky Way, Reeses	_   0	0	<b>⊗</b>	0	0	0	0	0	0		4	
Candy without chocolate (1 oz.)		10	(W)	Q	0	0	0	0	0		<b>6</b>	
Cookies, home baked (1)	10	10	(W)	Q	0	0	0	0	0	<u> </u> 000		
Cookies, ready made (1)	10	10	(W)	Q	Q	9	0	0	Q	<u> </u> 000		
Brownies (1)	10	10	(W)	Q	0	0	0	0	Q			
Doughnuts (1)	10	1 Ö	(W)	O	O	9	Q	Q.	Q			
Cake, home baked (slice)	18	10	(W)	<u>Q</u>	Ö	0	Q	O	Ŏ			
Cake, ready made (slice)	10	l Ö	(W)	Q	Q	0	Ŏ	0	Ŏ			
Sweet roll, coffee cake or other pastry, home baked (serving)	0	0	<b>⊗</b>	0	0	<b>©</b>	0	0		000	@ ©@  3 @@	
Sweet roll, coffee cake or other pastry, ready made (serving)	0	0	00	0	0	<b>©</b>	0	0	0	$O$ $\bigcirc$		<b>1</b>
Pie, homemade (slice)	0	10	l w	0	0	0	0	0	0			
Pie, ready made (slice)	Ö	ĬŎ	₩	Ŏ	Ŏ	0	ŏ	Ŏ	ŏ	<b>0</b> 00		
Jams, jellies, preserves, syrup, or honey (1 Tbs)	Ŏ	lŏ	l 🔞	ŏ	ŏ	<u></u>	ŏ	ŏ	ŏ			
Peanut butter (Tbs)	Τŏ	Tŏ	l 🔞	ŏ	ŏ	0	ŏ	ŏ	ŏ	000		
Popcorn (1 cup)	lò	ĬŎ	l 🔞	Ŏ	Ŏ	0	Ŏ	Ŏ	Ŏ	ŎŎŎ		
Nuts (small packet or 1 oz.)	0	Ō	(W)	Ŏ	Ŏ	(e)	Ŏ	Ŏ	Ŏ	ÖÖÖ		
Bran, added to food (1 Tbs)	0	0	(W)	0	Ō	0	Ŏ	Ō	Ŏ	000		
Wheat germ (1 Tbs)	0	0	00	0	0	(0)	0	0	0	000		
Chowder or cream soup (1 cup)	0	0	W W	0	0	(	0	0	0	000	@100	100
Oil and vinegar dressing, e.g. Italian (1 Tbs)	0	0	(W)	0	0	(0)	0	0	0		6 6	000
Mayonnaise or other creamy salad dressing (1 Tbs)	0	0	(W)	0	0	0	0	0	0	O 0 0 0	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	
Mustard, dry or prepared (1 tsp)	0	0	00	0	0	0	0	0	0		<b>®</b> 200	
Pepper (1 shake)	10	0	00	0	0	0	Ō	Ŏ	Ŏ		900	
Salt (1 shake)	0	0	8	0	0	0	0	O	0	000	000	000
4. How much of the visible fat on your meats do you		10. Ho	w mar	y teas	poons	of		Т			000	
remove before eating?	.	sug	gar do	you ad	d to y	our				@@	@@@	)@@
Remove all visible fat	fat		_		od eac	n day!					333	
Remove majority Remove none (Don't eat meat)		11. WI	cookir	a						(4) (4)	444	(A)
5. What kind of fat do you usually use for frying		oil	do you	u se?			Specif	v tyna	and bra		(G) (G)	
and sautéing? (Exclude "Pam"-type spray)	ŀ	12. WI			<u> </u>	-	Орсси	ytype	and bre		000	
Real butter Vegetable oil OLa	ord	col	d brea	kfast							000	
Margarine Ovegetable shortening	aru	cer	real do	you se? —	,						000	
			,		ľ		Specif	v tvpe	and bra		<u> </u>	
6. What kind of fat do you usually use for baking?	-	13 A	e the	re any	, othe	r imn				t you usu	ally	@
Real butter O Vegetable oil O La	ard	ea	t at le	east o	nce p	er we	ek?	. 1000	io tria	r you usu	апу	ĕ
Margarine Vegetable shortening		Inc	ludo f				a.u4:11a		4			0
		ho	rseradi	sh, par	rsnips,	rhuba	rb, rad	ishes,	fava b	m sauce, o eans, carr	ot iuice.	ĕ
7. What form of margarine do you usually use?		CO	conut,	avoca	do, ma	ango, p	papaya	, dried	d aprice	ots, dates,	figs.	<u></u>
○ None ○ Stick ○ Tub ○ Spread												<u></u>
O Low-calorie stick O Low-calorie tub	5	(Do	o not i	nclude	dry sp	oices a	and do	not li	st som	ething tha	t has	9999999
8. How often do you eat food that is fried at home?		be	en 118(6	24 III (I	he pre	vious s	ection	13./				
(Exclude the use of "Pam"-type spray)					that y					Jsual ing size	Servi per w	
O Daily O 4-6 times per week		(2)										
O 1-3 times per week	ek	(a)									*****	
9. How often do you eat fried food away from home? (e.g. french fries, fried chicken, fried fish)		(b)										
O Daily O 4-6 times per week		(c)										
O 1-3 times per week  O Less than once a week	ek	(d)										

## APPENDIX 8 Saliva Collection Instructions

### **COLLECTING SALIVA SAMPLES**

Participation in this part of the study involves collecting a morning saliva sample every day over the course of one menstrual cycle. While it is not necessary that collection be done at exactly the same time each day, we hope that you will find a way to fit sample collection into your daily routine, so that it will constitute the least possible burden to you.

- Begin collecting on the first day of your menstrual cycle (that is, when your menstrual bleeding starts, begin your collection the following morning). Collect a sample every day until the start of the next menstrual period. Please collect in the morning.
- Do not eat, drink, chew or smoke anything for *at least* 15 minutes before collecting a sample. Food (for example, coffee, egg) contamination can significantly distort hormone readings.
- Make sure your mouth is not bleeding for any reason before collecting a saliva sample, as even a tiny amount of blood can throw off the measurement of hormone levels. If your gums are bleeding, try rinsing your mouth with cold water, waiting several minutes, and trying again.
- Do not brush your teeth immediately before collecting a sample, since brushing can cause your gums to bleed.
- If you do happen to miss a day, just continue the collection the next morning.

### Instructions for saliva collection:

- Using the indelible pen provided, mark a tube (on the tape) with your initials and the time and the date of collection. Also, mark the sample tubes collected on the days of menstrual flow with an "M".
- Chew a piece of the gum provided for a few seconds to stimulate saliva flow (you may prefer to use only half a piece of gum). Then carefully fill the tube to the bottom of the tape with saliva. If you have difficulty getting the saliva into the tube on the first couple of tries, you may find it useful to look in the mirror while you are collecting. Please use only the gum provided, as this has been screened for use with our assays. It is not necessary to remove the gum from your mouth before collecting the sample. If you get a lot of bubbles in the tube, get rid of them by tapping the bottom of the tube a few times sharply on a hard surface, then continue to fill the tube with saliva to the tape.
- Cap the tube securely.
- Keep the collected samples in the container provided. The tubes have been pretreated with a special preservative and do not require refrigeration or freezing. The preservative used, sodium azide, is toxic if ingested. It is coated on the inner surface of the bottom of the tubes and does not represent a health hazard under normal collection conditions. However, please store the tubes out of the reach of small children and pets and do not re-ingest any saliva from the tube following collection.
- When your sample collection is completed make sure each tube is tightly capped, and then mail the tubes back to us in the container and the box the tubes were shipped in, using the Priority Mail stamp and address label provided.

## APPENDIX 9 Saliva Collection Log Form

Saliva Collection Log Form		
Twin Name:		
ID Number:		. •
Diagra autor the dates and time	s saliva was collected and	l days of menstrual flow.

Please enter the dates and times saliva was collected and days of menstrual flow. Please send this sheet to laboratory in the box with the tubes. Thank you.

Date of Collection	Time of Collection	Menstrual Flow? Yes/No
2.		
1. 2. 3.		
4.		
5.		
<i>5. 6.</i>		
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<i>30</i> .		
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31. 32. 33. 34. 35.		
<u>34.</u>	<u> </u>	
<i>5</i> 3.		